

PHYSIOLOGY AND ECOLOGY OF ORCHID MYCORRHIZAL FUNGI WITH REFERENCE TO SEEDLING NUTRITION

BY SARAH E. SMITH

Department of Botany, Cambridge University

(Received 5 April 1966)

SUMMARY

Orchid mycorrhizal fungi have been assumed to supply carbohydrates to the orchid seedlings with which they are associated. To do this they must be able to utilize a regular source of carbohydrate in the soil, and also to translocate soluble compounds to the orchids. Mycorrhizal *Rhizoctonia* spp. were shown to decompose filter paper cellulose in pure culture. Secondly, *R. repens* and *R. solani*, isolated from *Dactylorhiza purpurella*, were able to compete with other soil micro-organisms in the exploitation of cellulose buried in unsterilized soil.

Rhizoctonia repens was found capable of translocating ^{32}P and ^{14}C labelled compounds in an already established mycelium. Radioactivity could also be detected in infected seedlings if tracers were fed to the infecting hyphae some distance from the seedlings. This translocation from fungus to seedlings resulted in greater growth of the seedlings if cellulose was available to the infecting fungus than if no carbohydrates were supplied to either fungus or seedlings.

INTRODUCTION

This paper describes the results of an investigation into the part played by orchid mycorrhizal fungi in the germination and growth of seedlings of green orchids. Orchid seeds are very small and early growth is slow, so that there is a prolonged phase before the seedlings of green orchids become self-supporting by photosynthesis. The limited food reserves in the seeds are inadequate to support growth of the seedlings throughout this period. Organic materials for growth, as well as water and mineral nutrients, must be supplied from outside until the autotrophic stage is reached. *Cattleya* seedlings were successfully grown on sugar containing media in the absence of any mycorrhizal fungus by Knudson (1925). Results of this kind led him to believe that orchid seedlings always obtained sugars directly from the medium; he attributed any stimulation of growth in the presence of a mycorrhizal fungus (usually observed in the presence of insoluble carbohydrates) to the action of fungal enzymes on the carbohydrates in the medium, resulting in an increased concentration of sugars in the neighbourhood of the seedlings. He showed that other fungi besides mycorrhizal fungi could in some cases be active in this process.

On the other hand, Bernard (1904, 1909), Burgeff (1936) and others considered that the mycorrhizal fungi played a more direct part in the nutrition of seedlings. They believed that the fungi not only broke down complex carbohydrates in the medium (or soil) but also absorbed sugars and transported them to the hyphae within the orchid seedlings, whence they passed to the orchid tissues.

If the latter hypothesis is to hold, the mycorrhizal fungi must satisfy certain conditions. Not only must they be able to utilize complex carbohydrates (such as cellulose)

VISIT...

LANZAROTE
Caliente.COM

and thus tap the most abundant supply of carbohydrate in the soil, but also they must be able to transport nutrients (in a form available to the orchid seedlings) from one point to another in an already established mycelium. There is some evidence that orchid mycorrhizal fungi possess both these properties. Holländer (1932) has shown that many orchid fungi are capable of breaking down cellulose and lignin in pure culture, but there has been little work on the behaviour of these fungi in soil. It has recently been shown that the widespread pathogen *Rhizoctonia solani* Kühn acts as a mycorrhizal associate of *Dactylorhiza purpurella* (T. & T. A. Steph.) Vermeul. (Downie, 1957). Garrett (1962) has shown that an isolate of this fungus (pathogenic to swede) is capable of decomposing filter-paper cellulose, not only in pure culture but also in competition with other organisms in unsterilized soil. This finding led to my investigation of the cellulose-decomposing ability of a mycorrhizal isolate of *Rhizoctonia solani* and of other orchid mycorrhizal fungi, described in the first part of this paper.

There is some evidence that orchid mycorrhizal fungi are able to translocate nutrients to orchid seedlings. Beau (1920) reported that seedlings of *Spiranthes autumnalis* Rich. and *Orchis fragrans* Poll. would grow on damp glass when connected to a gelatin substrate by fungal hyphae only. If the mycelial connections between seedlings and medium were severed, growth of the seedlings ceased. Downie (1943) obtained similar results for *Goodyera repens* (L.) R. Br. However, Knudson (1925) found that a *Cattleya* species failed to germinate under somewhat similar conditions.

Several groups of workers have studied the ability of *Rhizoctonia solani* to translocate nutrients in an established mycelium. Lucas and Robinson (1963, personal communication) and Monson and Sudia (1963) have shown that this fungus can translocate compounds labelled with ^{32}P . Thrower and Thrower (1961) have shown that the same fungus can transport ^{14}C -labelled compounds in an established mycelium. King and Isaac (1964) have reported that *R. solani* can translocate ^{14}C labelled material at 40 μmin along the hyphae.

Schütte (1956) considered that *Armillaria mellea* (Vahl ex Fr.) Kummer, the mycorrhizal associate of *Gastrodia elata* (Kusano, 1911) and *Galeola septentrionalis* (Hamada, 1940), was able to translocate sugars and phosphorus because it could grow from a medium containing these compounds to a medium deficient in them.

The second part of this paper describes experiments on the ability of *Rhizoctonia repens* (Bernard), isolated from *Dactylorhiza purpurella*, to transport carbon and phosphorus compounds in an already established mycelium and also into the tissues of infected orchid seedlings.

ISOLATION OF THE FUNGI

The fungi used in the experiments were isolated from the roots of *Dactylorhiza purpurella* by Downie's (1959) method. The roots were washed in running water to remove adhering soil, cut into 5-mm lengths and surface sterilized with 0.1% mercuric chloride in 20% ethanol. After washing in four to five changes of sterile distilled water, the root segments were plated onto potato-dextrose agar (PDA) acidified to pH 4.5–5.0 with phosphoric acid. The Petri dishes were incubated at 22.5° C and any fungi growing from the root segments were subcultured onto PDA and identified as far as possible. Four isolates obtained in this way have been used in the experiments described in this paper. Two were of the *Rhizoctonia repens* type (isolates A and E) and two could not be properly identified and are referred to as *Rhizoctonia* species X. In addition, four *Rhizoctonia*

isolates from orchid plants (sent by Dr. G. Hadley, of the Botany Department, University of Aberdeen) were also used. Table 1 summarizes the origins and identifications of the fungi, together with results of germination experiments with the appropriate orchid seed, where these were carried out.

Table 1. *Fungal isolates used in the experiments*

	Isolate	Host	Origin	Mycorrhizal
<i>Rhizoctonia solani</i>	Sa (CMI 192120)	<i>Dactylorhiza purpurella</i>	Dr. Hadley	—
<i>R. repens</i>	A	<i>D. purpurella</i>	S.E.S.	+
<i>R. repens</i>	E	<i>D. purpurella</i>	S.E.S.	+
<i>R. repens</i>	R1 (CMI 94840)	<i>D. purpurella</i>	Dr. Hadley	—
<i>Rhizoctonia</i> sp. X	B	<i>D. purpurella</i>	S.E.S.	+
<i>Rhizoctonia</i> sp. X	D	<i>D. purpurella</i>	S.E.S.	—
<i>R. gracilis</i>	Pc.a (CMI 192113)	<i>Platanthera chlorantha</i>	Dr. Hadley	—
<i>R. goodyerae-repentis</i>	G.r.	<i>Goodyera repens</i>	Dr. Hadley	—

—, No test; +, stimulated growth of orchid seedlings.

Commonwealth Mycological Institute (CMI) culture numbers quoted where available.

RESULTS

Cellulose decomposition in pure culture

The method used to assess cellulolytic activity of the mycorrhizal fungi has been described by Garrett (1962) for *Rhizoctonia solani*. Dry-weight loss of filter paper cellulose during a period of incubation with a fungus in pure culture was used as a measure of cellulose decomposing ability in the absence of competition from other micro-organisms. Conical flasks (250 ml), each containing a wad of ten Whatman No. 3 filter papers (7 cm diameter, weighing approximately 6.7 g) moistened with 20 ml mineral nutrient solution (Garrett, 1962), were inoculated with a disk, 11 mm in diameter, cut from the growing margin of a colony on PDA. In most experiments eight flasks were used for each isolate and eight left uninoculated as controls. The flasks were incubated at 22.0° C for 6 weeks, after which the filter-paper wads, together with as much mycelium as possible, were removed from the flasks and dried to constant weight at 80° C. Dry-weight loss of inoculated wads was determined and compared with controls. It was found that air-dry wads of filter-paper absorbed water from the atmosphere during weighing. For this reason the wads were dried in tins of known weight which were closed before cooling and weighing. Several fungi showed only a very small amount of cellulose decomposition when this method was used. In further experiments with these fungi, both control and inoculated wads were washed with 30 ml distilled water before drying and weighing, in order to remove soluble breakdown products of cellulose. The loss of weight of control wads after washing corresponded with the calculated weight of minerals added to them. In one experiment, lasting 3 weeks, the washings from filter-paper wads colonized by *R. repens* (A) and *R. solani* (Sa) were deionized with Amberlite ion exchange resins (IR 45 and IR 120) and analysed chromatographically. The solvent used was ethyl acetate-acetic acid-water (14 : 3 : 3) run in a downward direction (Smith, 1960). Marker compounds (glucose, cellobiose, trehalose and mannitol) were run on each chromatogram, in parallel with the solution for analysis. The reagents used for detection of reducing sugar and polyol spots were silver nitrate (Trevelyan, Proctor and Harrison, 1950) and aniline and oxalic acid followed by resorcinol and hydrochloric acid.

Table 2 shows the loss in weight of filter-paper wads colonized by the isolates, after 6 weeks incubation. Loss of weight was ascribed to respiration of the sugars produced during enzymic breakdown of the cellulose. Sugars converted to mycelial dry weight or remaining in the medium do not therefore contribute to the weight loss in most experiments. The results for *R. repens* and *R. gracilis* shows the increased loss in weight obtained by removing soluble compounds from the wads by washing. Chromatographic analysis showed that sugars other than the immediate products of cellulose breakdown (glucose, cellobiose and oligosaccharides) were present in the washings. *R. repens* (A) gave trehalose as well as glucose (no other compounds were detected) and *R. solani* (Sa) gave cellobiose, mannitol and glucose. Trehalose may also have been present but could not be detected in the presence of cellobiose, which runs very close to trehalose in the solvent used. These additional compounds must have originated in the fungal hyphae.

Table 2. *Cellulose decomposition in pure culture*

	Isolate designation	Dry-weight loss	
		(mg)	(%)
<i>Rhizoctonia solani</i>	Sa	1008 ± 27	14.4
<i>R. goodyerae-repentis</i>	G.r.	400 ± 50	5.7
<i>Rhizoctonia</i> sp. X	B	262 ± 21	3.3
<i>Rhizoctonia</i> sp. X	D	147 ± 5	2.2
<i>R. repens</i>	A	44 ± 5	0.6
<i>R. repens</i>	R1	Not significant	
<i>R. repens</i>	E	40 ± 4	0.6
<i>R. repens</i> (washed)	A	216 ± 12	3.1
<i>R. repens</i> (washed)	R1	200 ± 4	2.9
<i>R. repens</i> (washed)	E	299 ± 7	3.2
<i>R. gracilis</i>	Pc.a.	Not significant	
<i>R. gracilis</i> (washed)	Pc.a.	155 ± 6	2.2

Dry-weight loss of filter-paper after 6 weeks incubation in the presence of a fungus.

Measurement of loss in dry weight due to respiration clearly underestimates the actual amount of cellulose breakdown, and may lead to insignificant results with fungi of weak decomposing ability (e.g. *R. repens*). Washing the wads gives a closer measure of the actual amount of breakdown but is not satisfactory for the comparison of different species, as washing would be likely to remove different amounts of soluble material from different fungi. Although the results for the different species cannot be directly compared, it is clear that all the isolates broke down cellulose to some extent.

Cellulose decomposition in unsterilized soil

The following method was used to study the competitive saprophytic ability of the isolates with respect to cellulose. The growth of the fungi into a non-nutrient medium (acid washed sand), from a food-base composed of a two parts, a PDA inoculum disk (16 mm diameter) and a column of unsterilized soil (8 g light loam from 179 Hills Road, Cambridge), was compared with growth from a similar food-base augmented by cellulose (70 mg) buried in the soil. The experiments were set up in open-ended glass tubes, 1.9 cm diameter and 20 cm long. A 17 cm column of air-dry acid-washed sand was brought to 100% moisture-holding capacity (m.h.c.) with distilled water. Eight grams of soil were then added to each tube and brought to 50% m.h.c. Half the tubes had two disks of damp Whatman No. 3 filter paper added midway along the soil column. The

tubes were each inoculated with a disk, 16 mm diameter, cut from an actively growing fungal colony on PDA placed on the surface of the soil. Uninoculated tubes, with and without added cellulose, were included in each experiment. All the tubes were capped at both ends with moisture-proof cellophane (British Cellophane Co., MSAT 600), and incubated at 22.0° C. Eight tubes were usually used for each treatment.

Mycelial growth into the sand column from the base of the soil was measured at weekly intervals for 5–6 weeks using the $\times 25$ magnification of a stereoscopic dissecting microscope. At the same time the furthest occurrence from the base of the soil column of sclerotia and mycelial aggregates produced in the sand by the inoculated fungi was measured. The presence of competing soil fungi was also recorded. *R. solani* (Sa), *R. repens* (A) and *Rhizoctonia* sp. X (B and D) were used in these experiments.

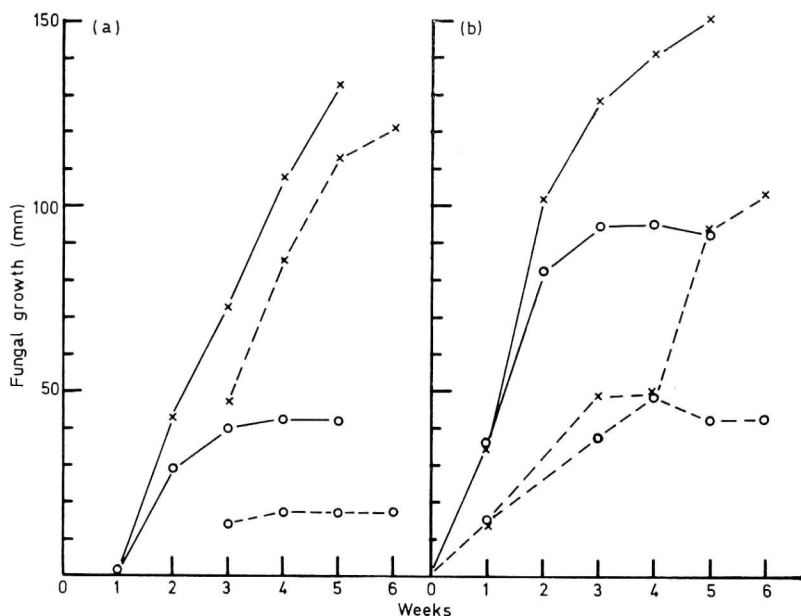


Fig. 1. Fungal growth in inoculated sand tubes. Growth of mycelium (—) and development of sclerotia or mycelial aggregates (---) in the presence (x) and absence (o) of added cellulose. (a) *Rhizoctonia repens*, (b) *R. solani*.

Dense mycelium of soil fungi always colonized the sand in the uninoculated tubes with added cellulose, but in those without cellulose only a few sparse hyphae were seen in some of the tubes. The addition of cellulose to the soil clearly stimulated the activity of cellulolytic soil fungi which were able to grow into the sand, and it was difficult to distinguish between young *Rhizoctonia* mycelium and sterile mycelium of the competing soil fungi. For this reason, measurements of mycelial growth in the tubes containing cellulose were not as reliable as those based on the development of mycelial aggregates (produced by *R. repens*) or sclerotia (produced by *R. solani*).

All the *Rhizoctonia* species colonized the soil and grew into the sand in tubes both with and without cellulose. Results for *R. repens* (A) and *R. solani* (Sa) are presented in Fig. 1. Both *R. repens* (A) and *R. solani* (Sa) were easy to recognize; the former produced

numerous small mycelial aggregates and the latter both brown hyphae and brown sclerotia. Not only was the mycelial growth of both fungi greater when cellulose was added to the tubes, but also mycelial aggregates and sclerotia were produced at a greater distance from the base of the soil column. The number of sclerotia produced by *R. solani* (Sa) was increased in the presence of cellulose. Both these fungi appeared to be able to compete successfully with the soil fungi and utilize the added cellulose as a food base. Garrett (1962) obtained similar results with a different isolate of *R. solani*. The competitive ability cannot be directly correlated with cellulolytic ability in pure culture; although *R. solani* decomposed cellulose rapidly *R. repens* was one of the slowest cellulose decomposers tested.

Inconclusive results were obtained with both isolates of *Rhizoctonia* sp. X.

Although mycelial growth of these isolates in the presence of cellulose was always greater than when no cellulose was added the differences were not statistically significant. Production of sclerotia by this fungus was erratic so that identification of the mycelium in the cellulose tubes was doubtful. These results emphasize that the method is only useful in the absence of effective fungal competitors, or when the inoculated fungi can be clearly recognized.

Translocation of radioactive compounds by R. repens

Translocation of compounds labelled with phosphorus-32 (^{32}P) and carbon-14 (^{14}C) was investigated using the split-plate method described by Lucas (1960). The plates were prepared by removing a strip of agar (0.5 cm wide) across the diameter of polystyrene Petri dish (8 cm diameter) containing 20 ml agar medium PDA or Knudson's B solution. As long as the plates remained dry this agar-free strip was found to act as an effective barrier to the diffusion of radioactive compounds from one side of the plate to the other. Experiments were carried out on *R. repens* in pure culture, both alone and in association with seedlings of *Dactylorhiza purpurella*. In experiments with the fungus alone Petri dishes were inoculated on one side with a disk of agar (5 mm diameter) cut from the margin of a growing colony of *Rhizoctonia repens*. In experiments involving both fungal hyphae and orchid seedlings, the seedlings themselves were used as inoculum of the fungus.

Seeds of *Dactylorhiza purpurella* were germinated and grown in the presence of *Rhizoctonia repens* on an agar medium composed of Knudson's B solution + 0.5% macerated cellulose (Whatman No. 3 filter paper ground in an MSE homogenizer). After 1-2 months seedlings about 1-3 mm long were transplanted to one side of the diffusion barrier in a split-plate.

Plates in both types of experiment were incubated in the dark at 22.5° C until the fungal hyphae had formed a bridge across the diffusion barrier from one side of the plate to the other. Labelled solutions were then added to a trough (3.0 × 0.5 cm) cut parallel to the diffusion barrier and 1 cm from it, on the opposite side of the plate to the inoculum disk or seedlings. Isotopes were obtained from the Radiochemical Centre, Amersham, Bucks. Carrier-free [^{32}P]orthophosphate in dilute HCl was diluted in 2% (0.14 M) KH_2PO_4 to give 20 $\mu\text{C}/\text{ml}$. Uniformly labelled [^{14}C]D-glucose (initially 50-150 $\mu\text{C}/\text{mm}$) was made up in 2% (0.11 M) glucose solution to give 20 $\mu\text{C}/\text{ml}$. A volume of solution, 0.25 ml, containing 5 μC was added to each plate.

In experiments with ^{14}C a small well (1 cm diameter) was embedded in the agar. At the time of addition of tracer the well was filled with 0.2 N sodium hydroxide (NaOH) and a piece of filter paper added, to absorb any $^{14}\text{CO}_2$ produced in respiration.

After different lengths of time the radioactivity present in samples of mycelium + agar and in seedlings was determined. Disks of agar + mycelium were removed from the plates with a No. 2 cork borer (5 mm diameter) as shown in Fig. 2.

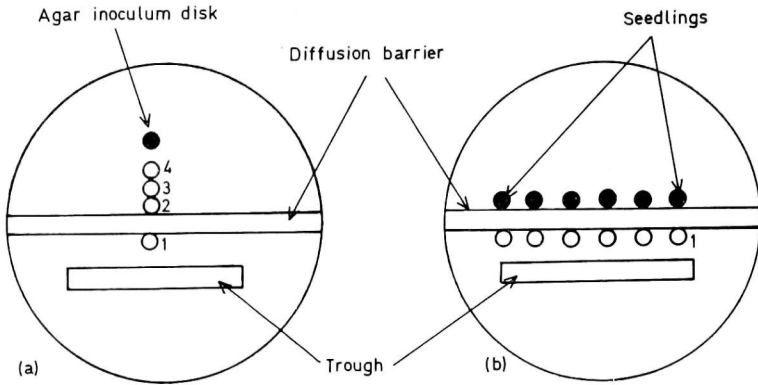


Fig. 2. Inoculation and sampling positions on split-plates used in translocation experiments. (a) With fungus alone, (b) seedling experiment. ●, Inoculation positions; ○, sampling positions.

Table 3. *Translocation of ^{32}P and ^{14}C labelled compounds by mycelium of Rhizoctonia repens*

	^{32}P		^{14}C	
Agar samples	Plate 1	Plate 2	Plate 3	Plate 4
1 (donor side)	109000	114000	6830	6140
2 } (receiver side)	555	1950	214	90
3 }	356	1120	76	38
4 }	325	400	31	15

Amount of tracer present (counts/min) in successive 5 mm diameter samples, 24 hours after application of [^{32}P]orthophosphate (plates 1 and 2) and [^{14}C]glucose (plates 3 and 4).

Table 4. *Translocation of ^{32}P and ^{14}C labelled compounds to seedlings of Dactylorhiza purpurella by hyphae of Rhizoctonia repens*

	^{32}P		^{14}C	
	24 hours		72 hours	
Agar (donor side)	22500	30800	23200	15400
Seedling (receiver)	30	33	32	166
				No count
				200
				19400
				144
	^{32}P		^{14}C	
	72 hours		72 hours	
Agar (donor side)	9651	11230	14633	10940
Seedling (receiver)	1046	955	709	926
				6099
				773

Amount of ^{32}P and ^{14}C present (counts/min) in seedlings, after application of [^{32}P]orthophosphate and [^{14}C]glucose. A single plate was used for each experiment.

Agar samples were prepared for counting by the method described by McCready (1958). The radioactivity in them gave a value for tracer present in the mycelium and any loss to the agar from it. Seedlings were picked off the surface of the agar, crushed in a few drops of 80% ethanol directly on a planchette and dried before counting.

A mica end window Geiger-Müller tube (Mullard MX 123) was used to count the radioactivity in the ^{32}P samples. A Panax scintillation counter, with a plastic scintillator, was used to count the ^{14}C samples. Results are expressed as counts per minute per sample, corrected for background radiation and counter dead time as appropriate. Counts for ^{32}P and ^{14}C samples cannot be directly compared, because of the different counting methods and the different radiations of the two isotopes.

It is clear from the results presented in Tables 3 and 4 that *R. repens* is able to transport both ^{32}P and ^{14}C labelled compounds across the agar-free diffusion barrier of a split-plate, in a previously established mycelium. It is also clear that tracer may appear in orchid seedlings as a results of this translocation. No significant movement of isotopes occurred across the diffusion barrier on plates on which the mycelium had been killed with propylene oxide just before the tracer solutions were added.

Translocation of carbon compounds to growing seedlings

The method used was based on that described by Schütte (1956). The experiments were set up as follows. Double dishes were made by sticking a 4-cm diameter Van Tieghem cell to the centre of a 9-cm Petri dish with araldite resin. The outer compartments of these dishes were filled with a mineral nutrient (Knudson's B solution) agar medium, with no added carbohydrate source. The inner compartments in half the double dishes were also filled with this medium. The inner compartments in the other half of the dishes were filled with a medium composed of Knudson's B solution plus 0.5% macerated cellulose (Whatman No. 3 filter paper, ground in an MSE homogenizer). Care was taken to prevent contact between the media in the two compartments, so that the glass wall of the Van Tieghem cell acted as a diffusion barrier between them.

Seeds of *Dactylorchis purpurella* or *D. praetermissa* were surface-sterilized with saturated calcium hypochlorite solution for 1 hour and spread on the surface of the agar in the outer compartment. *Rhizoctonia repens* was inoculated onto the centre of the inner compartment. The plates were incubated in the dark at 22.5° C. Uninoculated controls were included in each experiment.

The fungus grew over the wall of the Van Tieghem cell in the inoculated dishes and infected the seedlings in the outer dish. After different lengths of time those seedlings which had swollen and produced epidermal hairs were removed from the surface of the agar, fixed in formalin-acetic-alcohol and stored in 50% alcohol, to prevent changes in volume that would have occurred if the seedlings had been stored in water. The seedlings were measured microscopically, using a micrometer eyepiece, as soon as possible after removal from the agar. During the measurement of *Dactylorchis purpurella* seedlings it was recorded whether each seedling had turned brown or whether it had retained a healthy appearance. Results could then be expressed separately for each category.

The results (Table 5) show that growth of seedlings in the presence of *Rhizoctonia repens* was increased, as compared with uninoculated controls, whether cellulose was available to the fungus or not.

Table 5 also shows that while growth of seedlings of *Dactylorchis praetermissa* in the presence of cellulose continued after 7 weeks, in the absence of cellulose the seedlings made no growth between the seventh and fourteenth weeks after sowing. After 14 weeks seedlings of *D. purpurella* also were much larger in the presence of cellulose than in its absence (Table 6). When the two classes of seedlings 'brown' and 'healthy' were separated it was found that there was no significant difference in size of brown seedlings in the two treatments, but that 'healthy' seedlings were much larger in the presence of cellulose

than in its absence. It is possible that the brown seedlings in both treatments had been parasitized and killed by the fungus early in the experiment. The percentage of brown seedlings was not significantly different in the two treatments ($72 \pm 3.2\%$ and $62 \pm 6.5\%$ in the plates with and without cellulose respectively).

The use of seedlings as a measurable sink for translocated materials means that Schütte's double dish technique can be used for experiments on the translocation of

Table 5. *Growth of Dactylorhiza praetermissa seedlings in Schütte dishes*

	Not inoculated	Inoculated with <i>Rhizoctonia repens</i>	
		+ Cellulose	No cellulose
7 weeks			
No. of seedlings	20 (1)	20 (1)	20 (1)
Length (μ)	303 ± 10	572 ± 30	540 ± 48
Width (μ)	248 ± 10	462 ± 30	457 ± 28
14 weeks			
No. of seedlings	—	32 (1)	32 (1)
Length (μ)	—	697 ± 75	565 ± 54
Width (μ)	—	513 ± 44	412 ± 31

The number of dishes used for each sample is given in parentheses after the number of seedlings.

Table 6. *Growth of Dactylorhiza purpurella seedlings in Schütte dishes after 14 weeks*

	Not inoculated	Inoculated with <i>Rhizoctonia repens</i>	
		+ Cellulose	No cellulose
Total			
No. of seedlings	14 (1)	797 (4)	865 (3)
Length (μ)	248 ± 9	820 ± 52	625 ± 43
Width (μ)	206 ± 10	517 ± 35	444 ± 25
'Healthy'			
No. of seedlings	—	226 (4)	324 (3)
Length (μ)	—	1170 ± 119	800 ± 72
Width (μ)	—	692 ± 44	519 ± 39
'Brown'			
No. of seedlings	—	571 (4)	532 (3)
Length (μ)	—	471 ± 7	450 ± 17
Width (μ)	—	343 ± 37	371 ± 15

The number of dishes used for each sample is given in parentheses after the number of seedlings.

compounds through an established mycelium. This is not the case if, as in Schütte's (1956) experiments, the ability of a fungus to grow from a nutrient to a deficient medium is used as a criterion of translocating ability. Schütte's method does not distinguish between transfer of compounds as a result of hyphal growth and translocation in an established mycelium (Lucas, 1960).

DISCUSSION

If orchid mycorrhizal fungi are to be able to supply carbohydrates, either directly or indirectly, to orchid seedlings, they must be able to live independently either as soil saprophytes or as parasites of organisms other than orchids.

Many of the fungi have been shown to break down pectin (Pérombelon and Hadley, 1965), cellulose and lignin (Holländer, 1932) in pure culture. The list of orchid fungi able to decompose cellulose in pure culture has been extended in this investigation. In addition several fungi have been shown to compete successfully with other organisms for carbohydrate sources buried in the soil. Examples are *Armillaria mellea*, whose behaviour as a colonizer of buried wood blocks has been described by Garrett (1960), and *Rhizoctonia solani* and *R. repens* described in this paper. Further evidence also suggests that other orchid fungi are capable of living saprophytically in the soil. Downie (1943) has shown that *R. goodyerae-repentis* has a wider distribution in pine wood soils than the orchid (*Goodyera repens*) from which it is usually isolated. Similarly, Curtis (1937, 1939) showed that species of fungi isolated from different orchids were not specific to particular orchid species, but were characteristic of the habitats in which the individual plants grew. This suggests that the distribution of the fungi may be independent of the orchids.

Orchid mycorrhizal fungi pathogenic to host plants other than their orchid associates include *Armillaria mellea* and *Rhizoctonia solani*.

It is possible that the activities of orchid mycorrhizal fungi (and other soil fungi) in the neighbourhood of orchid seedlings may make soluble carbohydrates transiently available to the latter, according to the hypothesis of Knudson. However, it is difficult to accept any theory of orchid seed germination and growth which requires the maintenance of relatively high concentrations of sugars in the soil. The easy availability of these compounds to many soil organisms should argue against the possibility that a permanent source of sugars would be available to orchid seedlings in the soil. In pure culture, however, a single fungal species grown with orchid seeds may produce sufficient soluble carbohydrate in the medium to allow germination and growth of seeds in the absence of any infection by the fungus (Knudson, 1925). Nevertheless, the pure culture experiments described in this paper cannot be interpreted in this way. In the experiments on growth of seedlings of *Dactylorhiza praetermissa* and *D. purpurella* in double dishes the carbohydrate source was separated from the seedlings, so that the only connections between them were fungal hyphae of *Rhizoctonia repens*. This fungus is capable of breaking down cellulose and also of translocating ^{32}P and ^{14}C labelled compounds through the hyphae. The tracer experiments do not show whether or not the labelled compounds are retained within the infecting fungal hyphae or whether they are available for growth of the seedlings. However, increased growth of seedlings when cellulose was supplied to the fungus indicates that carbohydrates are made available to the seedlings. It is reasonable to suppose that an accumulation of dry matter occurred in the seedlings as a result of translocation, and that there was a net transfer of carbon compounds in the hyphae. Further experiments are in progress on the incorporation of ^{14}C into both fungal and orchid tissue when [^{14}C]glucose is supplied to infecting fungal hyphae.

The results of the translocation experiments are in accord with those obtained by Beau (1920) for *Spiranthes autumnalis* and *Orchis fragrans*; he showed that mycelial connections between a gelatin medium and seedlings in distilled water were necessary if seedling growth was to occur. The results described in this paper show that such mycelial connections must be involved in supplying nutrients to the seedlings, as changes in the amount of carbohydrate available to the fungus influences the growth of the seedlings. The results are contrary to those of Knudson (1925), who found that seedlings of a *Cattleya* species adhering to the glass walls of culture tubes would not grow even

when attached to the medium by fungal hyphae. Infected seedlings on the medium grew well. Differences in the amount of water available to the seedlings may explain these apparently contradictory results. Water is required for the initial swelling of the embryo and rupture of the seed coat before any germination will occur. It is probable that less water was available to the seeds in Knudson's experiments than to seeds in comparable treatments in experiments of Beau and myself. This suggestion is supported by Knudson's results; his infected seedlings on the walls of the tubes swelled less than uninfected seedlings on the surface of the agar medium. Seeds in Beau's experiments were in distilled water and in my experiments were spread on an agar medium so that water was available to all the seedlings.

It seems most logical to suppose that organic compounds, having been translocated to the orchid seedlings through the infecting fungal hyphae, pass directly into the orchid cells, as suggested by Burgeff (1936). Coils of hyphae are present within some of the infected orchid cells, so that there is a large area of contact between the two organisms, across which such a transfer could occur (Harley, 1959). It would be interesting to investigate the structural relationships between the internal hyphae and the orchid cytoplasm both in these cells and in those in which 'digestion' of the fungal hyphae is taking place. This digestion may also play a part in the transfer of nutrients from fungus to orchid, as Burgeff believed. However, it is unlikely that digestion is strictly necessary for the transfer to occur. Harley (1959) has pointed out that transfer of materials from fungal sheath to root tissues occurs readily in beach mycorrhizas, where the fungal hyphae rarely penetrate the root cells.

ACKNOWLEDGMENTS

The work described in this paper formed part of a thesis submitted for the degree of Ph.D. in the University of Cambridge. I would like to thank the trustees of the Brooks Fund; Newnham College, Cambridge; and the Science Research Council, all of whom have contributed to my financial support.

I am most grateful to my supervisor, Dr. S. D. Garrett, for his advice and encouragement during this work and also for helpful criticism of the manuscript.

REFERENCES

- BEAU, C. (1920). Sur le rôle trophique des endophytes d'orchidées. *C. r. hebd. Séanc. Acad. Sci., Paris*, **171**, 675.
- BERNARD, N. (1904). Recherches expérimentales sur les orchidées. *Revue gén. Bot.*, **16**, 405.
- BERNARD, N. (1909). L'évolution dans la symbiose. Les orchidées et leur champignons commensaux. *Annls Sci. nat.*, series 9, **9**, 1.
- BURGEFF, H. (1936). *Samenkeimung der Orchideen*. Gustaf Fischer, Jena.
- CURTIS, J. T. (1937). Non-specificity of orchid mycorrhizal fungi. *Proc. Soc. exp. Biol. Med.*, **36**, 43.
- CURTIS, J. T. (1939). The relation of specificity of orchid mycorrhizal fungi to the problem of symbiosis. *Am. J. Bot.*, **26**, 390.
- DOWNIE, D. G. (1943). The source of the symbiont of *Goodyera repens*. *Trans. Proc. bot. Soc. Edinb.*, **33**, 383.
- DOWNIE, D. G. (1957). *Corticium solani*—an orchid endophyte. *Nature, Lond.*, **179**, 160.
- DOWNIE, D. G. (1959). The mycorrhiza of *Orchis purpurella*. *Trans. Proc. bot. Soc. Edinb.*, **38**, 16.
- GARRETT, S. D. (1960). Rhizomorph behaviour in *Armillaria mellea* (Fr) Quél. 3. Saprophytic colonisation of woody substrates in soil. *Ann. Bot.*, **24**, 275.
- GARRETT, S. D. (1962). Decomposition of cellulose in soil by *Rhizoctonia solani* Kühn. *Trans. Br. mycol. Soc.*, **45**, 115.
- HAMADA, M. (1940). Studien über die Mykorrhiza von *Galeola septentrionalis* Reichb. f. Ein neuer Fall der Mykorrhizabildung durch intraradicale Rhizomorphen. *Jap. J. Bot.*, **10**, 151.
- HARLEY, J. L. (1959). *The Biology of Mycorrhiza*. Leonard Hill, London.

- HOLLANDER, S. (1932). Ernährungs-physiologische Untersuchung an Pilzen saprophytische lebender Orchideen. Dissertation, Würzburg (reported in Burgeff, 1936).
- KING, M. K. & ISAAC, P. K. (1964). The uptake of Glucose-6-T and Glycine-2-T by *Rhizoctonia solani* Kuhn. *Can. J. Bot.*, **42**, 815.
- KNUDSON, L. (1925). Physiological study of the symbiotic germination of orchid seeds. *Bot. Gaz.*, **79**, 345.
- KUSANO, S. (1911). *Gastrodia elata* and its symbiotic association with *Armillaria mellea*. *J. Coll. Univ. Tokyo*, **4**, 1.
- LUCAS, R. L. (1960). Transport of phosphorus by fungal mycelium. *Nature, Lond.*, **188**, 763.
- MCCREADY, C. C. (1958). A direct plating method for the precise assay of Carbon-14 in small liquid samples. *Nature, Lond.*, **181**, 1406.
- MONSON, A. M. & SUDIA, T. W. (1963). Translocation in *Rhizoctonia solani*. *Bot. Gaz.*, **124**, 440.
- PÉROMBELON, M. & HADLEY, G. (1965). Production of pectic enzymes by pathogenic and symbiotic *Rhizoctonia* strains. *New Phytol.*, **64**, 144.
- SCHÜTTE, K. H. (1956). Translocation in the fungi. *New Phytol.*, **55**, 164.
- SMITH, I. (1960). *Chromatographic and Electrophoretic Techniques*, Vol. 1: Chromatography. Heinemann, London.
- THROWER, S. L. & THROWER, L. B. (1961). Transport of carbon in fungal mycelium. *Nature, Lond.*, **190**, 823.
- TREVELYAN, W. E., PROCTOR, D. P. & HARRISON, J. S. (1950). Detection of sugars on paper chromatograms. *Nature, Lond.*, **166**, 444.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.